

Physiology of the Pulmonate Reproductive Tract:¹

Location of Spermatozoa

in Isolated, Self-Fertilizing Succinid Snails

(with a Discussion of Pulmonate Tract Terminology)

BY

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(1 Plate; 1 Text figure)

INTRODUCTION

CAIN IN 1956 CONCLUDED HER PAPER ON fertilization in *Lymnaea* as follows: "The problems of the location of fertilization and the function of the seminal receptacle still remain unresolved." Despite the great strides in biology during the past 17 years, this statement is as true today as it was then, and it could be extended to cover several other aspects of the pulmonate reproductive tract. Familiarity with the elusive nature of these problems, and with the importance of snail breeding to medical malacology led me to carry out this study.

The physiological context of these problems is as follows. The simultaneous hermaphroditism typical of pulmonate snails is accompanied, in many species, both basommatophoran and stylommatophoran (see PATTERSON, 1970), by the ability to self-fertilize. The possibility of parthenogenesis has been widely investigated and has been ruled out in all species studied (LARAMBERGUE, 1939). Although there is controversy about the situation in particular species (CAIN, 1956), it is probable that in normally breeding populations of snails, copulation followed by cross-fertilization is the mode of reproduction most commonly employed. The work of IKEDA & MURA (1934, *Bradybaena similis simpsoni* (Férussac)) crossing banded and unbanded snails, together with CAIN's (1956, *Lymnaea stagnalis appressa* Say, 1817) work,

extended recently by RICHARDS (1970, 1973, *Biomphalaria glabrata* (Say, 1818)), in which albino and wild-type basommatophorans have been raised in isolation and then allowed to cross-copulate, has given us valuable data on dominance of allosperms and their longevity in the 'foreign' reproductive tract.

Some enigmatic problems are raised by these data. (1) By what means do allosperms dominate over auto-sperms in the fertilization of ova? (2) Where are the allosperms stored between copulation and fertilization? — an interval which may last up to 116 days in *Lymnaea stagnalis* (Linnaeus, 1758) (CAIN, 1956), and more than a year in certain helicids (LARAMBERGUE, 1939).

In attempting to answer the second of these questions one is faced with histological data which further extend the problem. In normal adult pulmonates grown in mass culture (individuals not isolated), mature sperms are found possibly in 3 places — the seminal vesicles (ovotestis duct), the spermatheca, and the gonad, though it is doubtful whether sperms found in the latter are quite mature since it is thought that immediately upon maturation pulmonate sperms leave the gonad and enter the seminal vesicles where they are stored (AUBRY, 1954). It has been consistently shown (see LARAMBERGUE, 1939; BAYNE, 1970) that sperms are broken down in the spermatheca³. Therefore, apparently only the seminal vesicles remain as a possible storage site for allosperms between the time that they are received at copulation and the time they are used in fertilization. Within the seminal vesicles

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³ Breakdown consists most noticeably of a loss of the sperm tail (BAYNE, 1970). IKEDA (1937) considered this to be equivalent to a maturation after which the sperms could fertilize. Most reports, however, indicate that sperms are tailed at the time of fertilization (LARAMBERGUE, 1939b, FERROT, 1939).

numerous autosperms are stored; auto- and allosperms are microscopically indistinguishable, so that confirmation of this as an hypothetical storage site is not possible by routine microtechnique. The genera *Succinea* (RIGBY, 1965) and *Anguispira* (GUGLER, 1964) are exceptions in that they are reported to possess receptacula seminis, at the base of the ovotestis duct, in which oriented allosperms may be found.

In attempting to answer the first of the two questions above, two theories may be proposed: a) spermatozoa are not capable of fertilization until they have mingled with secretions from the pallial reproductive tract⁴ – that is, they require capacitation or physiological maturation; or b) ova are not capable of being fertilized until they reach the site at which allosperms are situated at the time of fertilization – that is, the absence of fertilization during the passage of ova through the dense mass of sperms in the seminal vesicles is due to the nature of the ova, not the nature of the sperms (IKEDA, 1937; PERROT, 1937; HORSTMANN, 1955). A third theory, proposed by PEREZ (1889), that the sperms in the seminal vesicles are re-sorbed prior to each oviposition, is unacceptable in light of more recent reports of ova and sperms being seen together in the seminal vesicles of ovipositing pulmonates (PERROT, 1937; ABDEL-MALEK, 1954; DUNCAN, 1956; this study).

Before a full understanding of the functioning of the pulmonate reproductive tract is possible, a great research effort is necessary involving many experiments on many different species. On account of two reproductive features, *Succinea grosvenori* (Lea, 1864) was chosen for the first of these experiments. Firstly, succinid snails possess 2 diverticula, thought to be receptacula seminis, in the region of the ovotestis duct-albumen gland junction (Figure 1) (RIGBY, 1965). Secondly, *S. grosvenori* has been shown to reproduce in isolation (PATTERSON, 1970). The disputed relationships of the family Succinidae (BURCH & PATTERSON, 1968) added further interest.

If sperms must undergo some physiological maturation, possibly by encountering some secretion(s) in the pallial reproductive tract prior to self-fertilization, then a study of the location of sperms in the reproductive tracts of isolated, self-fertilizing succinids should give some valuable data when examined in snails at various stages of reproductive activity. The receptacula seminis might be con-

⁴ It might seem that a more meaningful theory would postulate that this capability is acquired in the process of sperm exchange during copulation (GRATIOLET, 1851), but such a statement would leave no room for the self-fertilizing which can occur if there is no copulation, *e. g.*, in isolated individuals, and possibly in aphallic populations like *Bulinus truncatus*.

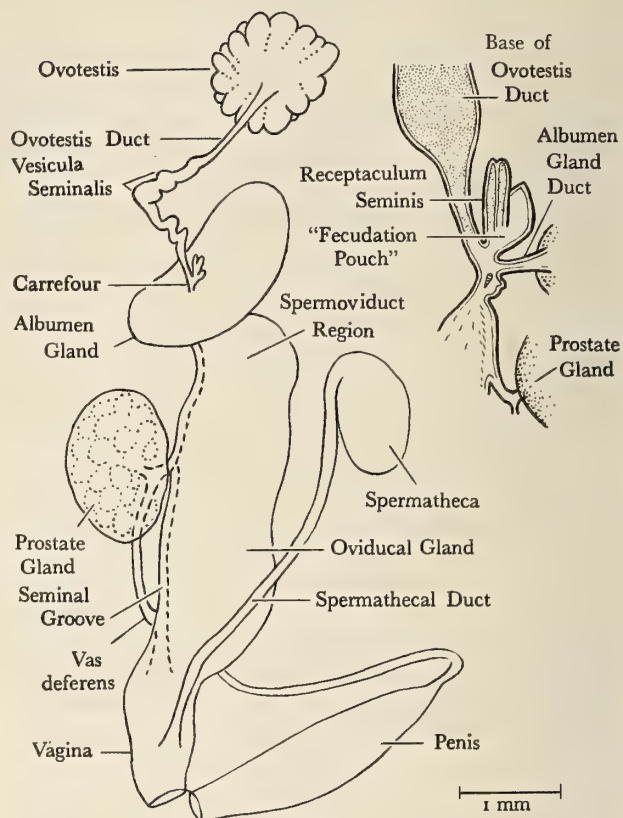


Figure 2

sidered as likely sites for such maturation, but it has also been suggested (IKEDA, 1929; SOOS, 1935) that maturation occurs in the spermatheca (= bursa copulatrix). One other feature of reproductive physiology might be further elucidated by such a study; that is the role of the 'prostate,' a gland whose function has not been adequately documented in any pulmonate (BAYNE, 1967).

Reports by LAMS (1910, *Arion empiricorum* Férussac), CRABB (1927, *Lymnaea stagnalis appressa*), AUBRY (1955, *L. stagnalis*) and LANZA & QUATTRINI (1964, *Vaginulus borellianus* (Colosi) and *Laevicaulis alta* Férussac, 1812) that fertilization takes place within the gonads of self-fertilizing pulmonates are consistent with the recent report by BRISSON (1968) that in *Bulinus truncatus* Audouin intra-gonadal fertilization and development can be induced by removal of more anterior parts of the repro-

ductive tract. These reports would suggest that a physiological maturation of sperms and eggs does not require a mixing with secretions of the pallial reproductive tract. Instead a situation seems apparent in which a barrier to self-fertilization is eventually overcome by gametes if allosperms are not received over a long period. Certain species exhibit a lower fecundity if grown in isolation (IKEDA & MURA, 1934; LARAMBERGUE, 1939; LAVIOLETTE, 1954; BAYNE, 1970); many, however, do not suffer this apparent disadvantage (CRABB, 1927; IKEDA, 1937; LANZA & QUATTRINI, 1964).

In this paper we are concerned with the mechanism whereby self-fertilization is normally avoided, but remains a possible alternative to cross-fertilization. We acknowledge that this flexible barrier to self-fertilization may be seated in the sperm or in the egg, or in both. The problems are very elusive of investigation. We are therefore asking, if the barrier is seated in the sperm:

- i) must the fertilizing sperm (be they auto- or allosperms) be capacitated as in mammals?
- ii) if so, what secretions are involved?

Data gathered during this research provide the first evidence that prostatic secretion may be important in the process of self-fertilization, in addition to its documented role in copulation, and its possible role in egg mass formation (BAYNE, 1967).

MATERIALS AND METHODS

Two species were used in the expectation that any existing intra-generic variation would be apparent: *Succinea grosvenori*, 9th generation in laboratory culture, and *S. unicolor* Tryon, 1866, 8th generation in laboratory culture. Six-day old specimens of *S. grosvenori* and 5-day old specimens of *S. unicolor* were placed individually into separate, large Petri dishes prepared for culture as described by PATTERSON (1970). The snails were fed lettuce, powdered chalk, Cerophyll⁵ (a finely ground preparation of a mixture of cereal grasses) and water. Daily attention was necessary in order to maintain adequately clean and healthy conditions. At the ages shown in the Table specimens were killed by submersion in alcoholic Bouin's fixative. They were serially sectioned in paraffin wax at 7 μ m and stained with azan-Mallory (PANTIN, 1964). The sections were all examined for the locations of sperms and for observations on the state of the various reproductive glands. Viable eggs were obtained from the specimens kept for the longest times.

⁵ No longer commercially available.

Table 1

Events in the Development and Functioning of the Reproductive Tracts of Both Succinid Species

No.	Date fixed	Shell		Comments (s. v. = seminal vesicle; pros = prostate; alb. = albumen)
		Age (wks)	length (mm)	
<i>Succinea grosvenori</i>				
2	6/VI 1969	4	4.2	Maturing ova and sperm; s. v. + sperm; primordial tract
3	6/VI	4	3.6	Sp-tids and oogonia, no s. v. or sperm; primordial tract
4	10/VI	4.5	3.8	Well developed gonad; s. v. + sperm; tract glandular
6	13/VI	5	3.7	Sp-gonia + sp-tids, no sperm, very primordial tract
7	13/VI	5	2.0	Sperm (few) in s. v.; primordial tract
10	17/VI	5.5	5.5	Sperm in s. v.; glands starting to accumulate secretion
11	23/VI	6.5	7.5	Sperm in s. v.; tract well developed
13	26/VI	7	5.0	Died just prior to fixation; sperm in s. v.; glands mature
14	28/VI	7.3	7.3	1 batch eggs laid; sperm in s. v.; alb and pros secreting; few sperm in enlarged fecundation pouch
15	30/VI	7.5	?	3 batches eggs laid (26/VI, 28/VI; 30/VI); sperm in s. v.; prostate secretion in seminal groove
<i>Succinea unicolor</i>				
1	6/VI	4	3.8	Possible gonia
5	10/VI	4.5	5.0	Sp-gonia and sp-tids, no sperm; primordial tract
8	13/VI	5	5.2	Oocytes and sperm; small s. v.; primordial tract
9	17/VI	5.5	5.5	Oocytes and sperm; small s. v.; primordial tract
12	23/VI	6.5	6.8	Sperm in s. v.; glands maturing
16	2/VII	7.7	8.2	Sperm in s. v.; some free alb. gd. secretion; pros poorly developed
17	11/VIII	13.5	8.0	9 eggs laid, ova in s. v.; no sperm in carrefour complex; alb. gd. + secretion in lumen; pros + secretion

RESULTS

Maturation of the Reproductive Tract

As with all pulmonate organ systems, the maturation of the reproductive system lacks clear-cut physiological stages. The development of a mature reproductive tract is a gradual process which begins early and is subject to considerable variations in rates between individuals.

Specimens of *Succinea grosvenori* matured a little earlier in this study than *S. unicolor*. Four weeks after hatching, stages of both male and female gametes were found in the young gonad of *S. grosvenori*, and in one specimen the ovotestis duct contained a small mass of sperms, thus representing the early seminal vesicle. At this age the pallial reproductive tract was merely a cord of undifferentiated cells. Whereas 2 specimens at 5 weeks still had primordial tracts, one specimen at 4½ weeks had a glandular tract, though not yet fully mature. One specimen at 5 weeks did not even contain any sperm; the other had a few in the seminal vesicle. The only specimen fixed at 5½ weeks contained sperms in the seminal vesicle, and the tract was becoming glandular, with accumulation of secretion beginning. All specimens aged 6½ weeks or more had histologically mature reproductive tracts.

Succinea unicolor at 4 weeks contained the earliest rudiments of a gonad containing only undifferentiated 'gonia.' No tract rudiment was recognized. By 4½ weeks such a rudiment was discernible, and spermatogenesis was occurring but no sperms were present in the ovotestis duct. At 5 and 5½ weeks both oocytes and sperms were present in the gonad and a few sperms were in the ovotestis duct; the tract was, however, still rudimentary and non-glandular. Maturation of the pallial glands was in progress at 6½ weeks. By 7.7 weeks the tract was mature. The late laying of this species (13½ weeks) may have been due to a requirement for more water than is necessary for normal growth, as oviposition occurred a few hours after an increase in the amount of free water in the container. (In the Basommatophora, a change of water often stimulates oviposition.)

Location of Spermatozoa

In all mature and maturing snails prior to egg laying, sperms were restricted to the gonads and seminal vesicles. In one specimen which had laid eggs, a few sperms were present in the 'fecundation pouch.' In snails raised in isolation sperms were absent, both prior to and after egg laying, from the receptacula seminis and from the spermatheca.

Two specimens of *Succinea grosvenori* which had been grown in communal containers were sectioned. Sperms were found in the receptacula seminis of both; poor fixation precludes a definite statement as to the contents of the spermatheca of one of these. The other contained filamentous material resembling sperm remains.

Other Observations

The conditions of the 'prostate' gland and the spermatheca of isolated specimens were significant. The spermatheca did not remain empty in the absence of copulation; in mature isolated individuals it always contained a large bolus of apparent detrital mucoid material which took a blue colour with Mallory III. The source of this material is unknown. The spermathecal duct is very narrow and not capable of much enlargement; it seems likely that the epithelium lining the spermatheca secretes some of the material in the bolus.

The most surprising finding was that in snails which had laid eggs the 'prostate' glands had always been actively secreting (Figure 1a). Prostatic secretion was present in the lumina of the gland and was found to fill the ciliated 'seminal' groove of the lower oviduct of specimen 15 all the way to the vagina.

Four ova were present in the ovotestis of specimen 17. They were located within the mass of sperms in the seminal vesicle (Figure 1b). It is noteworthy that these ova were not near the wall of the seminal vesicle where they might have been transported by epithelial cilia. A similar situation has been reported in *Physa fontinalis* (Linnaeus 1758) (DUNCAN 1958).

DISCUSSION

The results presented here are not entirely consistent with other published data on pulmonates. IKEDA (1937) reported that even in isolated *Philomycus bilineatus* (Rafinesque), a stylommatophoran, sperms were found in the spermatheca; but COLTON's (1912, *Lymnaea columella* Say, 1817) and LARAMBERGUE's results (1939, *Bulinus contortus* Michaud, 1889 = *B. truncatus* Audouin), together with those of AZEVEDO, COSTA FARO & GONCALVES (1959, *Biomphalaria glabrata*) – all basommatophorans – like my own showed no sperms in the spermathecae of virgins. Also, fertilization did not occur within the gonad as has been described for certain other species (LAMS, 1910; CRABB, 1927; AUBRY, 1955; LANZA & QUATRINI, 1964). These inconsistencies may be taken as indicative of a considerable interspecific variation in the physiology of the pulmonate reproductive tract.



Figure 1a



Figure 1b

Indications from this study indicate that if capacitation occurs, neither the receptacula seminis nor the spermatheca are involved. The active secretion of the 'prostate' reported here supports the idea that this organ may be functional not only in copulation but also in oviposition or fertilization or both (BAYNE, 1967). The presence of prostatic secretion in the ciliated channel of the oviduct indicates that it may be concerned with any capacitation which may occur.

Although the terms 'fertilization pocket' and 'fecundation pouch' are widely applied to a small chamber which opens into the carrefour complex, actual evidence of its function is scanty. RIGBY (1965) stated that it aggregates sperm packets and expels them into the male part of the spermoviduct. She also stated that it is the site of fertilization, though this was not based on actual observations. The presence here of sperms in one isolated individual which had laid eggs in the present study is further circumstantial evidence for its role as a fertilization pocket.

The absence of sperms from the receptacula seminis of isolated snails and their presence there in snails grown communally also amounts to strong but circumstantial evidence that these 2 blind-ending pockets are indeed the storage sites for allosperms. The equivalent site in basommatophorans is problematical (ALAPHILIPPE, 1959), there being no equivalent receptaculum seminis (RIGBY, 1965); it is expected that further research will elucidate this problem.

Reproductive Tract Terminology

A review of the literature on the morphology of the pulmonate reproductive tract clearly indicates that a confusion of terminology exists. This confusion can be particularly upsetting for investigators new to the area. In this short résumé suggestions are made, on the basis of (probable) function, as to the preferable names for all the major glands and ducts, excluding those of the terminal reproductive complex. It is hoped that this discussion will be beneficial to the field through its contribution to a standardized terminology.

The gonad, which produces both male and female gametes, is widely referred to as the hermaphrodite gland. While the organ is indeed hermaphrodite, this name does not specifically state that it produces gametes of both sexes, therefore the term *ovotestis* is preferred. The duct along which gametes pass from the ovotestis to the top (proximal end) of the pallial (glandular) reproductive tract is a hermaphrodite duct. However, since part of

the pallial tract (spermoviduct) of the Stylommatophora is also hermaphrodite in function, the term *ovotestis duct* is preferable for this duct in the Stylommatophora. In all pulmonates part of this duct is modified as a seminal vesicle.

The word 'carrefour' (French = crossroads, intersection) is acceptable as applicable to the junction of ducts at the base (distal end) of the ovotestis duct. It must be emphasized, however, that the term is not applicable to any specific structure, but rather to this general area, which is exceedingly complex and of great functional importance (HOLM, 1946; BRETSCHNEIDER, 1948; WU, 1972). The small size, delicate nature and complex relationships of the component ducts, pockets, and grooves make it difficult to unravel their true relationships. However, recently WALTER (1968, 1969) has published excellent accounts of the carrefours in *Lymnaea catascopium* (Say) and 3 species of *Bulinus*, and WU (1972) has made additional contributions for the genus *Bulinus*. However, in WALTER's words (1969) "The terminology expresses theoretical sequential stages in reproductive processes inferred solely from the structural relationships." In the absence of any functional studies his terminology must be accepted. Hopefully the validity of such terms as 'insemination chamber' and 'seminal reservoir' will be either proven or disproven as a result of future studies.

The terms *vesicula seminalis* (seminal vesicle), *receptaculum seminis* (seminal receptacle), *bursa copulatrix* and *spermatheca* are particularly subject to being confused. A *vesicula seminalis* is properly defined as an organ which stores autosperms. The term is therefore properly applied to that part of the pulmonate ovotestis duct which is more or less modified for sperm storage in the adult snail or slug. A *receptaculum seminis* is an organ which stores sperms received at copulation; it is therefore properly applied to the bilobed structure of that name in *Succinea* and in *Anguispira* (GUGLER, 1964). This term should not be used to refer to the spermatheca or bursa copulatrix discussed below. Most pulmonates evidently lack receptacula seminis as distinct organs. The term *bursa copulatrix* suggests a role in copulation and should not be used to designate the pocket which is usually attached to the vagina or atrium of pulmonates. Instead this pocket, or stalked pouch, should, at present, be referred to as the *spermatheca*. I say 'at present' because the role of this organ is as yet unclear. In pulmonates which produce a spermatophore (*i.e.*, certain stylommatophorans), the spermatheca probably dissolves this and releases the sperms, but this may not be its sole function. There are substantive data (Fretter, personal communication) which suggest that the spermatheca, which may